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ORIGINAL ARTICLE

2,3-Diarylxanthones as Potential Inhibitors of Arachidonic Acid Metabolic Pathways

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Abstract—In response to an inflammatory stimulus, arachidonic acid (AA), the main polyunsaturated fatty acid present in the phospholipid layer of cell membranes, is released and metabolized to a series of eicosanoids. These bioactive lipid mediators of inflammation arise physiologically through the action of the enzymes 5-lipoxygenase (5-LOX) and cyclooxygenases (constitutive COX-1 and inducible COX-2). It is believed that dual inhibition of 5-LOX and COXs may have a higher beneficial impact in the treatment of inflammatory disorders rather than the inhibition of each enzyme. With this demand for new dual-acting anti-inflammatory agents, a range of 2,3-diarylxanthones were tested through their ability to interact in the AA metabolism. *In vitro* anti-inflammatory activity was evaluated through the inhibition of 5-LOX-catalyzed leukotriene B_4 (LTB_4) formation in human neutrophils and inhibition of COX-1- and COX-2-catalyzed prostaglandin E_2 (PGE_2) formation in human whole blood. The results showed that some of the studied arylxanthones were able to prevent LTB_4 production in human neutrophils, in a concentration-dependent manner. The xanthone with a 2-catechol was the most active one ($IC_{50} \sim 9$ μM). The more effective arylxanthones in preventing COX-1-catalyzed PGE_2 production presented IC_{50} values from 1 to 7 μM, exhibiting a structural feature with at least one non-substituted aryl group. All the studied arylxanthones were ineffective to prevent the formation of PGE_2 catalyzed by COX-2, up to the maximum concentration of 100 μM. The ability of the tested 2,3-diarylxanthones to interact with both 5-LOX and COX-1 pathways constitutes an important step in the research of novel dual-acting anti-inflammatory drugs.

KEY WORDS: xanthones; 5-LOX; COX-1; COX-2; human neutrophils; human whole-blood assay.

INTRODUCTION

Inflammation is the natural response of the organism to tissue damage, most of the times arising from physical or chemical irritations, infections caused by a pathogen, or other injuries. It is also part of a complex physiological protective response to harmful stimuli in a body's attempt to heal itself, through the elimination of the initial cause of cell injury, removing necrotic cells and tissues, and initiating the process of repair. The inflammatory response is induced by chemical mediators produced locally by damaged cells at the site of inflammation or derived from circulating inactive precursors (typically synthesized by the liver) that are activated at the referred site [1]. There are two types of chemical mediators: cell-derived mediators that include histamine and serotonin (preformed mediators in secretory granules) and mediators synthesized as needed such as arachidonic acid (AA) metabolites (leukotrienes, prostaglandins, and platelet-activating factor), cytokines, and nitric oxide; and plasma-derived mediators which include complement activation system, kinin

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system, and coagulation/fibrinolysis system [2]. These mediators are involved in the activation of resident cells such as endothelial and epithelial cells and/or the recruitment and activation of inflammatory cells such as macrophages, monocytes, and neutrophils [1]. Two of these proinflammatory mediators are leukotrienes and prostaglandins that arise via arachidonic acid metabolism. The first step is the hydrolysis of this 20-carbon polyunsaturated fatty acid present in the phospholipid layer of cell membranes, catalyzed by the enzyme phospholipase A_2. Then, free AA can be metabolized by several lipoxygenases (LOXs), leading to the formation of a series of hydroxy acids and leukotrienes. In mammalian tissues, 5-LOX is generally found in cells of myeloid origin, like polymorphonuclear leukocytes, activated macrophages, and mast cells, and it is the main enzyme implicated in inflammatory and allergic disorders. The metabolism of AA can also involve the formation of prostaglandins through the action of membrane protein cyclooxygenase (COX, also known as prostaglandin H synthase). Two isoforms of COX are implicated in the inflammatory process: COX-1, which is constitutively expressed within most tissues and responsible for the normal physiological production of prostaglandins, and COX-2, that although absent in most normal tissues is highly induced by several inflammatory and mitogenic cells and is responsible for the overproduction of prostaglandins during inflammation [3].

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most common medicines used to restrain the characteristic signs of an inflammatory process. Nonetheless, drawbacks such as gastrointestinal disorders and renal damage limit their use. An alternative strategy is the use of selective COX-2 inhibitors that decreases these effects but is associated to cardiac implications [4]. Thus, many efforts have been made in order to develop the so-called dual-acting anti-inflammatory drugs able to inhibit both 5-LOX and COXs, maintaining the activity of classical NSAIDs while avoiding their side effects [5, 6].

Xanthones (9*H*-xanthen-9-ones) are an important class of oxygenated heterocyclic compounds widespread in nature. The pharmacological properties of both natural and synthetic xanthones (*e.g.*, anti-inflammatory, antimalarial, antioxidant, and antitumour activities) are associated to different substituents in different positions of their main core [7–12]. Aryl groups linked to xanthones are scarce in natural sources, but several publications reporting their synthesis and/or biomedical potential have been highlighted in literature and reviewed in [13]. In fact, the hydroxylated 2,3-diarylxanthones studied in the present work were already described as capable of scavenging oxygen and nitrogen reactive species [14, 15] and inhibiting lipid peroxidation [16]. As so, they can be seen as excellent candidates to act as modulators of the inflammatory process. The present investigation was undertaken to evaluate the interaction of a range of 2,3-diarylxanthones in the AA metabolism regarding their interference in the 5-LOX enzyme activity, namely through the inhibition of leukotriene B_4 (LTB_4) production in human neutrophils, and COX-1 and COX-2 enzyme activities, specifically through the inhibition of prostaglandin E_2 (PGE_2) production in human whole blood.

MATERIAL AND METHODS

Reagents, Chemicals, and Equipment

All chemicals and solvents used were of analytical grade, obtained from commercial sources, and used as received. Acetylsalicylic acid, arachidonic acid (AA), calcium ionophore (A23187), chremophor® EL, dimethyl sulfoxide (DMSO), gentamicin sulfate, Hank's balanced salt solution (HBSS), lipopolysaccharides from *Escherichia coli* 026:B6 (LPS), methanol, nordihydroguaiaretic acid (NDGA), and trypan blue solution 0.4% were obtained from Sigma-Aldrich Co. LLC (St. Louis, USA). The thromboxane synthase inhibitor (TXBSI) was synthesized as previously described [17]. The "Leukotriene B4 Enzyme Immunoassay (EIA) Kit" was obtained from Cayman Chemical (Ann Arbor, MI). The "PGE2 EIA Kit" was obtained from Enzo Life Sciences (Lausen, Switzerland). 2,3-Diarylxanthones **1–3** (Fig. 1) were synthesized according to a previously described procedure [18].

A multimode microplate reader (Synergy HT, BIOTEK) with temperature control capacity was used to perform the spectrophotometric readings in all the assays.

5-LOX—Neutrophil Assay

Isolation and Treatment of Human Neutrophils

Following informed consent, neutrophils were isolated from venous blood collected from healthy human volunteers, by antecubital venipuncture, into K_3EDTA vacuum tubes. The isolation of the human neutrophils was performed by the density gradient centrifugation method as previously described [19]. Cell viability and cell yield were evaluated by the trypan blue exclusion method, using a Neubauer chamber and an optic microscope with ×40 magnification. HBSS was used as the incubation medium and the isolated neutrophils' suspensions were kept on ice

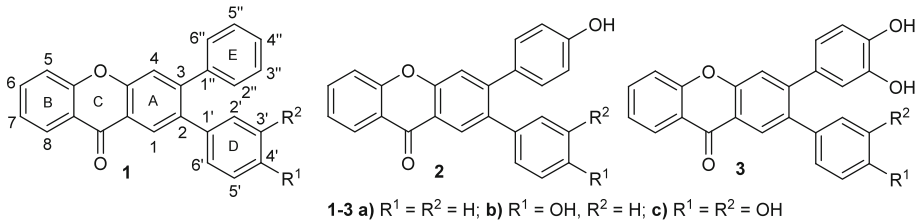


Fig. 1. Chemical structures of the tested 2,3-diarylxanthones **1**–**3**.

until use. Neutrophils' suspensions (3.5×10^6 cells/mL) in HBSS were placed in 96-well microplates (140 μL/well) at 37 °C for 10 min to equilibrate. The tested xanthones (1.0–20 μM), dissolved in a (9:1) mixture of HBSS:DMSO, were then added and pre-incubated for 10 min. The cells were subsequently incubated with A23187 (5 μM) and AA (10 μg/mL) for 8 min. The reactions were stopped by the addition of cold methanol. Samples were subsequently centrifuged at 13,000×*g* for 5 min at 4 °C and the supernatants were collected and stored at −20 °C until use [20]. The quantity of solvents used did not have inhibitory effects and neither affected the cellular viability.

Determination of LTB_4 Production in Human Neutrophils

The amount of LTB_4 in the collected supernatants was measured using the above mentioned commercial EIA kit, according to the manufacturer's instructions. The 5-LOX inhibitor, NDGA (1 μM), was used as positive control. The results were expressed as the percent inhibition of control LTB_4 production. At least three independent experiments were performed to each assay.

COXs—Human Whole-Blood Assay

COX-1 Assay

Following informed consent, venous blood was collected from healthy human volunteers, by antecubital venipuncture, into heparin-Li^+ vacuum tubes. The human whole-blood assay to assess the COX-1 inhibition was performed as previously reported [21, 22]. Collected blood (500 μL) was placed in microtubes and incubated in a water bath at 37 °C with TXBSI (1 μM) and the tested xanthones (0.312–100 μM) dissolved in DMSO for 15 min. Then, the A23187 (12.2 μg/mL) was added and the mixture was incubated for 15 min, allowing the triggering of COX-1 activity. The reactions were stopped putting the samples on ice for 5 min, followed by centrifugation at 1000×*g* for 20 min at 4 °C. The supernatants were then collected and stored at −20 °C until use. The quantity of solvents used did not have inhibitory effects and neither affected the cellular viability.

COX-2 Assay

The human whole-blood assay to assess the COX-2 inhibition was performed as previously described, with modifications [22, 23]. Collected blood (800 μL) was placed in six-well plates and incubated for 15 min in a humidified incubator at 37 °C with TXBSI (1 μM), acetylsalicylic acid (10 μg/mL), and the tested xanthones (1–100 μM), dissolved in a (1:10) mixture of DMSO:(chremophor/ethanol 1%). Here, TXBSI reduces the amount of LPS needed and its incubation period. Then, LPS (10 μg/mL) was added and the mixture was incubated for 5 h, allowing the activation of COX-2. The reactions were stopped by adding DPBS-gentamicin buffer (1 mL) to the samples and placing them on ice for 10 min. Subsequently, the samples were centrifuged at 1000×*g* for 15 min at 4 °C and the supernatants were collected and stored at −20 °C until use. The quantity of solvents used did not have inhibitory effects and neither affected the cellular viability.

Determination of PGE_2 Production in Human Whole Blood

The amount of PGE_2 in the samples (thawed plasma supernatants) was measured using the above mentioned commercial EIA kit, according to the manufacturer's instructions, as an indicator of COX-1 and COX-2 activities. Indomethacin (1 μM) and celecoxib (10 μM) were used as positive controls for COX-1 and COX-2 assays, respectively. The results were expressed as the percent inhibition of control PGE_2 production. At least three independent experiments were performed to each assay.

Statistical Analysis

Statistical analyses were calculated using the GraphPad Prism 6 software (GraphPad Inc., La Jolla,

CA). The results were expressed as the percent inhibition of control LTB_4 production [mean ± standard error of the mean (SEM)]. IC_{50} values were calculated from the curves of percent inhibition of control LTB_4 production or PGE_2 production *versus* compound concentration. Statistical comparison between groups was estimated using the one-way analysis of variance (ANOVA), followed by the Bonferroni post hoc test. In all cases, p values lower than 0.05 were considered as statistically significant.

RESULTS

Inhibition of LTB_4 Production in Human Neutrophils

All the tested xanthones were able to inhibit the 5-LOX–LTB_4 production induced by A23187 and AA in human neutrophils, in a concentration-dependent manner (Fig. 2). It was only possible to calculate the IC_{50} value for the most active compounds, and regarding these results, the order of potencies found was as follows: **1c** (9.2 ± 4.1 μM), **1b** (11.6 ± 4.6 μM), **3a** (13.5 ± 0.6 μM), and **3b** (13.7 ± 2.1 μM) (Table 1).

At 20 μM, the highest tested concentration, xanthones **1b** and **3b** exhibited higher inhibitory rates of 71.1 ± 6.8 and 68.5 ± 5.8%, respectively. Xanthones **1c** and **3a** were also quite active with percentages of inhibition of 62.9 ± 5.8 and 60.7 ± 2.6%, respectively, for 20 μM. The other tested xanthones presented activities lower than 50% up to 20 μM.

Overall, from compounds of group 1, at 20 μM concentration, derivative **1b** (71.1 ± 6.8%) was the most active, followed by **1c** (62.9 ± 5.8%) and **1a** (40.5 ± 6.9% of inhibition). All xanthones from group 2 were generally less active than the other tested xanthones, being derivative **2c** the best of its group (60.7 ± 2.6% of inhibition). From group 3, xanthone **3b** (68.5 ± 5.8%) was the most active one, followed by **3a** (60.7 ± 2.6%) and **3c** (46.7 ± 1.2% of inhibition).

NDGA was used as positive control and showed an inhibitory activity of 47.7 ± 3.5%, for a concentration of 1 μM (Table 1).

Inhibition of PGE_2 Production in Human Whole Blood

In what concerns inhibition of COX-1–PGE_2 production stimulated by A23187, the most active compounds were compounds **2a**, **1a**, and **1b** (IC_{50} = 0.8 ± 0.2, 1.7 ± 0.4, and 2.8 ± 0.4 μM, respectively) (Table 1). Compounds **3a**, **2b**, and **1c** were also effective inhibitors with IC_{50} values of 6.6 ± 1.1, 15.8 ± 3.3, and 29.5 ± 4.7 μM, respectively.

The results in terms of percentage of inhibition, to 10 μM concentration, are in accordance with the IC_{50} values found. Thus, it is possible to establish two groups from the studied xanthones (Fig. 3). The first group includes the most active compounds, with inhibitory effects higher than 50% (for a concentration of 10 μM), in the

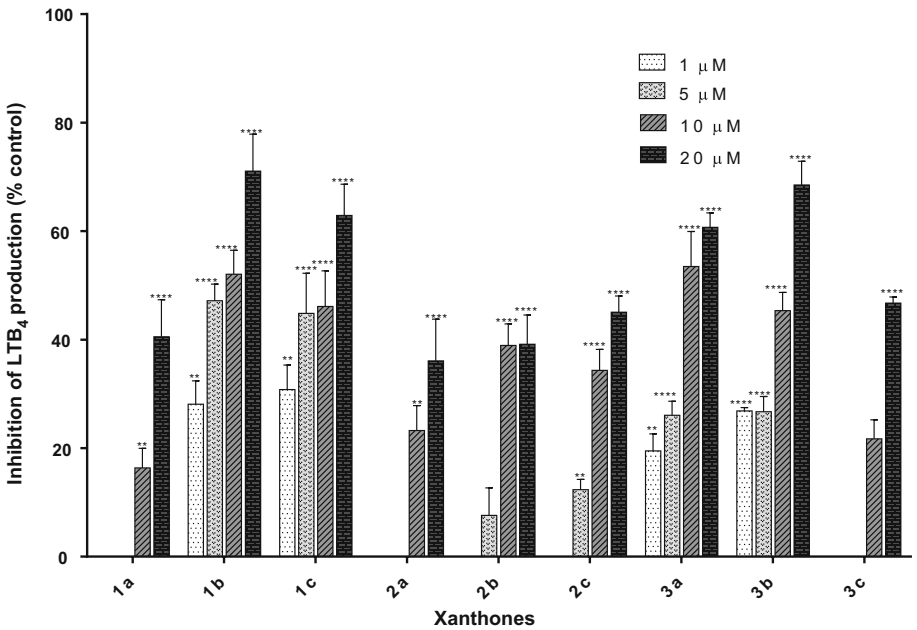
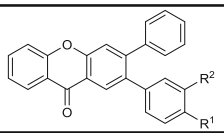
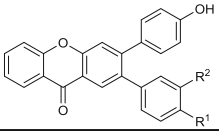
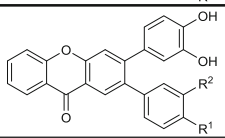


Fig. 2. Inhibition of LTB_4 production induced by A23187 and AA in human neutrophils by the tested xanthones **1–3**, determined by EIA. Each value represents the mean ± SEM of at least three experiments. ****$P < 0.0001$, **$P < 0.01$, compared to the stimulated control (A23187/AA).

Table 1. Inhibitory Effects (IC_{50} μM, mean ± SEM) of the Studied Xanthones **1–3** on the 5-LOX–LTB_4 Production in Human Neutrophils and COX-1–PGE_2 Production in Human Whole Blood. Each Study Corresponds to at Least Three Experiments

Compounds		R^1	R^2	IC_{50} (μM) ± SEM	
				5-LOX	COX-1
1a		H	H	ND	1.7 ± 0.4
1b		OH	H	11.6 ± 4.6	2.8 ± 0.4
1c		OH	OH	9.2 ± 4.1	29.5 ± 4.7
2a		H	H	ND	0.8 ± 0.2
2b		OH	H	ND	15.8 ± 3.3
2c		OH	OH	ND	ND
3a		H	H	13.5 ± 0.6	6.6 ± 1.1
3b		OH	H	13.7 ± 2.1	ND
3c		OH	OH	ND	ND
Positive controls					
NDGA				$47.7 \pm 3.5^{1\mu M*}$	ND
Indomethacin				ND	$89.7 \pm 2.4^{1\mu M*}$

ND not determined
[a] The values represent the percentage of inhibition ± SEM for the highest tested concentration (in superscript)

following order of potencies: **1a** (91.4 ± 2.1%) > **2a** (89.7 ± 6.1%) > **1b** (78.8 ± 7.1%) > **3a** (65.8 ± 6.8%). In the second one are placed the xanthones with activities lower than 50%, for 10 μM concentration. Compounds **2b** (38.2 ± 4.9%) and **1c** (22.1 ± 7.2%) were the most active of this second group, being the other derivatives ineffective to inhibit the COX-1–PGE_2 production at this concentration. Nevertheless, xanthones **3b** and **3c** showed an inhibitory

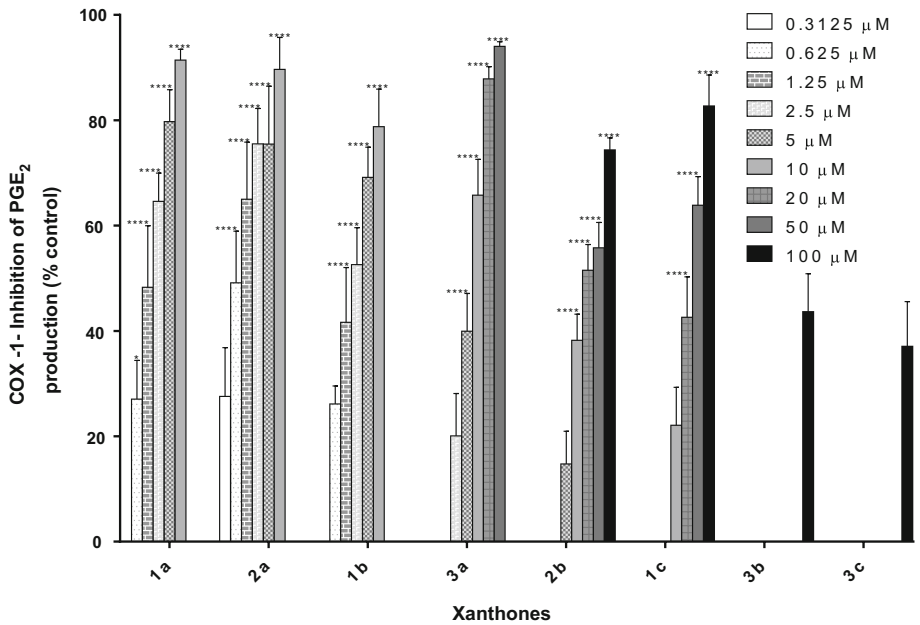


Fig. 3. Inhibition of COX-1 PGE_2 production induced by A23187 in human whole blood by the xanthones **1–3** determined by EIA. Each value represents mean ± SEM of at least three experiments. ****$P < 0.0001$, *$P < 0.1$, compared to the stimulated control (TXBSI/A23187).

activity of 43.7 ± 7.2 and 37.1 ± 8.5%, respectively, for 100 μM, the highest tested concentration (Fig. 3).

Overall, among all the tested compounds, in a 10 μM concentration, xanthones of group 1 were generally more active than the corresponding xanthones from the other groups, derivative **1a** being the best of its group (91.4 ± 2.1% inhibition). From group 2, xanthone **2a** was the most active one (89.7 ± 6.1% inhibition), followed by **2b** (38.2 ± 4.9% inhibition), whereas **2c** was not active, up to the highest tested concentration, 100 μM. Xanthone **3a** (65.8 ± 6.8% inhibition) was the only effective inhibitor from group 3, the less active group of all the studied xanthones (Fig. 3). Indomethacin was used as positive control and showed an inhibitory activity of 89.7 ± 2.4%, for a concentration of 1 μM (Table 1).

None of the studied xanthones **1–3** were able to inhibit COX-2–PGE_2 production induced by LPS in human whole blood, up to the highest tested concentration, 100 μM. Selective COX-2 inhibitor celecoxib (10 μM) reached a 75.4 ± 7.2% inhibitory effect.

DISCUSSION

The inhibition of 5-LOX pathway by the studied 2,3-diarylxanthones **1–3** are reported here for the first time. All the studied compounds proved to be effective inhibitors of LTB_4 production induced by A23187 and AA in human neutrophils at the concentration of 20 μM (in a range of inhibition from 36 to 71%). Generally, compounds of group 1 (non-substituted 3-phenyl ring) were the most active inhibitors while compounds of group 2 were the less active ones (possessing a 3-phenol group). An important structural feature for the higher inhibitory effect seems to be the presence of 2- or 3-catechol groups. Xanthone **1c** (substituted with 2-catechol group and non-substituted 3-phenyl ring) presented an IC_{50} value of around 9 μM. In fact, the presence of a catechol moiety in a wide variety of flavonoids has been described as vital for the inhibition of prooxidant enzymes like LOX [24, 25]. Moreover, Sadik *et al.* also pointed out that the inhibitory effect on 15-LOX enzyme by several flavonoids possessing a catechol unit correlates inversely with the number of other hydroxy groups in their skeleton [25]. The excessive number of hydroxy groups lowers the hydrophobicity of the compound and restrains their access to the active site of the enzyme. This feature can be an explanation for the lower inhibitory activity of compound **3c** (the highly substituted tested xanthone) when compared to the other xanthones of group 3.

Few studies have highlighted the potential anti-inflammatory action of a range of xanthones; however, it is a hard task to make a comparison of results since different experimental conditions and enzyme sources are used. As examples of studies with natural xanthones, 1,3,6,7-tetrahydroxyxanthone, a mangiferin aglycone known as norathyriol **4a** (Fig. 4), suppressed A23187-induced LTB_4 formation in rat neutrophils (IC_{50} = 2.3 ± 0.2 μM) and in blood taken from pretreated mice to about 20% of corresponding control values [26]. Aqueous extract of *Mangifera indica* L. and the glucosylxanthone mangiferin **4b** (Fig. 2) isolated from this extract have also been tested for the A23187-induced LTB_4 release in J774 murine macrophage [27] and RAW264.7 macrophage [28] cell lines. The purified compound showed a higher inhibitory effect (IC_{50} = 2.1 μg/mL) than the extract (IC_{50} = 26.0 μg/mL) for J774 murine macrophages whereas mangiferin **4b** at 10 μg/mL presented 68.8% of inhibition and the extract at 10 μg/mL showed 41.0% of inhibition of LTB_4 production, for RAW264.7 macrophages. Crockett *et al.* isolated a furanoxanthone **5** (Fig. 4) from the roots of *Hypericum perforatum* and evaluated the inhibition of 5-LOX-catalyzed LTB_4 formation in polymorphonuclear leukocytes. The results indicated an inhibition of 98.46 ± 0.59% at 50 μg/mL (146 μM) and an IC_{50} of 10.2 μM [29].

Several mechanisms can be associated to the inhibition of LTB_4 production by flavonoids and phenolic compounds. It can include the inhibition of the enzyme phospholipase A_2 and consequently the release of AA, the inhibition of enzyme LTA_4 hydrolase, the blockade of 5-lipoxygenase-activating protein for leukotriene biosynthesis, and even direct inhibition of 5-LOX [20]. Werz and Steinhilber proposed that LOX inhibitors can be divided into redox-active compounds, iron-ligand inhibitors with weak redox-active properties, and non-redox-type inhibitors [30]. Examples of redox-active compounds are NGDA, coumarins, and flavonoids, but unfortunately, the former ones presented lack of selectivity in most cases and were rapidly metabolized. Hydroxamic acid and *N*-hydroxyurea derivatives act as iron-chelating inhibitors, but their hydroxamate group is easily metabolized *in vivo* and showed unfavorable pharmacokinetic properties. The classification of non-redox-type inhibitors is associated to the lack of redox activity of the drugs themselves but does not exclude that their inhibitory effect is influenced by the redox state of the target 5-LOX. Several compounds tested by oral administration proved to be selective to inhibit leukotriene biosynthesis in human leukocytes and whole blood but without success to inhibit their synthesis at sites of chronic inflammation [24, 30]. As far as we know, no

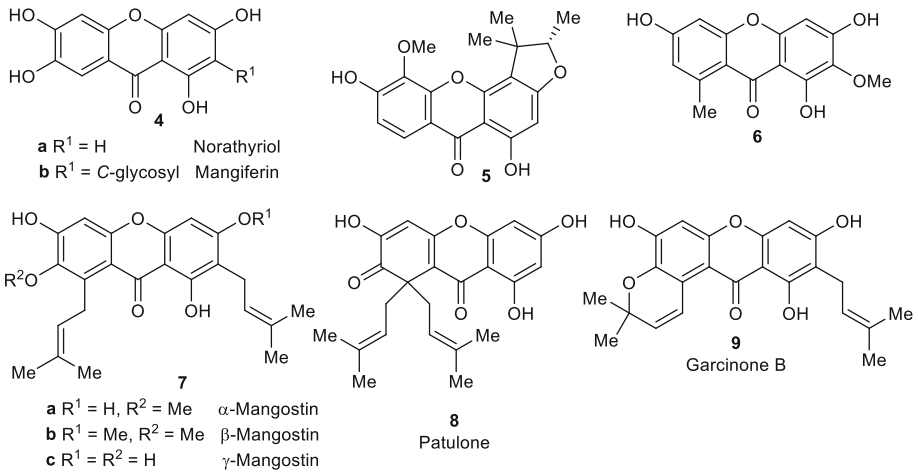


Fig. 4. Chemical structures of xanthones already studied in LOX and COX assays.

mechanism underlying the effect of xanthones on 5-LOX pathway has been proposed. We can presume that similar to phenolic compounds, xanthones act as redox 5-LOX inhibitors. In fact, this type of 5-LOX inhibition is pointed to the hydroxyxanthone norathyriol **4a** by the results of inhibition of human recombinant 5-LOX activity in a cell-free system [26]. Previous studies suggested that phenolic inhibitors can act at the 5-LOX catalytic domain by reducing the active iron from catalytically active Fe(III) to the inactive Fe(II) form [31] or by modulation of the hydroperoxide tone [32].

The results from the COX assays showed that the studied 2,3-diarylxanthones were only able to inhibit the COX-1–PGE_2 production stimulated by A23187 in human whole blood, in a concentration-dependent manner, except compound **2c** that was not active up to 100 μM, the highest tested concentration. The most effective inhibitors in each group of xanthones tested were those non-substituted in ring D, specifically **1a**, **2a**, and **3a** with IC_{50} values from 1 to 7 μM. Similar to the results of 5-LOX inhibition, increasing the number of hydroxy groups seems to be disadvantageous in preventing the production of PGE_2 catalyzed by COX-1. Indeed, xanthones **2**c (not active) and **3b** (holding three hydroxy groups) and xanthone **3c** (with four hydroxy groups) did not reach an IC_{50} at all. These data are corroborated by Ribeiro *et al.* that previously found in structural-similar compounds, the less substituted flavonoids were those presenting a higher inhibitory activity [22].

This is the first paper that reports the inhibition of COX-1 activity by 2,3-diarylxanthones, but there are a few studies that explore the inhibitory capacity of other substituted xanthones using different experimental conditions. Thus, Hsu *et al.* tested the inhibitory effect of ram seminal vesicle COX-1 activity in a range of 18 mono, di, tri, and tetraoxygenated xanthones [26]. Norathyriol **4a** (Fig. 4) was the most active compound ($IC_{50} = 16.2 \pm 1.5$ μM), with even higher inhibitory potency than the other three tetrahydroxyxanthones tested. Mono, di, and trihydroxyxanthones were less active than norathyriol, at least with fourfold lower potency. From this study, we can also state that norathyriol **4a** showed a similar inhibition of COX-1 and COX-2 activities and a higher efficiency to inhibit human recombinant 5-LOX [26]. The inhibition of ram seminal vesicle COX-1 activity was also examined for furanochromone **5** [29] and trihydroxylated xanthone **6**, isolated from the bulbs of *Ledebouria ovatifolia* [33] (Fig. 4). These compounds displayed minor inhibitory effects of $18.41 \pm 6.50\%$ at 50 μg/mL (146 μM) and $44 \pm 11\%$ at 10 μM, respectively, although xanthone **6** was selective for COX-2 at 10 μM. γ-Mangostin **7c**, a xanthone present in the fruit hull of mangosteen, and its related compound patulone **8** exhibited a similar effect but in higher extent than garcinone B **9** (Fig. 4) on PGE_2 release stimulated by A23187 in C_6 rat glioma cells [34, 35]. The *in vitro* enzyme assays showed that γ-mangostin **7a** prevented, in a concentration-dependent manner, both COX-1 ($IC_{50} = 0.8$ μM) and COX-2 ($IC_{50} = 2$ μM) activities [34] as well as patulone **8** (Fig. 4) had the ability to prevent the enzymatic activity of COX-1 [35].

Although none of the studied 2,3-arylxanthones **1–3** were able to prevent the COX-2–PGE_2 production stimulated by LPS in human whole blood, there are several reports highlighting the preventive effects of several xanthones against COX-2 activity. Garrido *et al.* studied the LPS-interferon gamma-induced PGE_2 release by mangiferin **4b** in J774 murine macrophage cell lines that

showed a high inhibitory activity with an IC_{50} value of 17.2 μg/mL [27]. Using RAW264.7 macrophage cells, the inhibitory potency of α-mangostin **7a** (IC_{50} = 13.9 μg/mL) and γ-mangostin **7c** (IC_{50} = 13.5 μg/mL) was also very significant [36] while β-mangostin **7b** proved to be a selective inhibitor of COX-2 (53.0 ± 6.0% inhibition at 20 μg/mL), with a slight effect on COX-1 activity (17.1 ± 1.0% inhibition at 20 μg/mL) [37]. A similar behavior was observed for furanoxanthone **5** tested in a concentration of 50 μg/mL where the results pointed to a moderate inhibition of COX-2 activity (36.15 ± 4.68%) and a slight inhibition of COX-1 activity (18.41 ± 6.50%) [29]. This short results seem to indicate that xanthones bearing oxygenated substituents and lipophilic carbon chains are good candidates as COX-2 inhibitors.

CONCLUSION

In this study, for the first time, a range of 2,3-diarylxanthones showed to suppress LTB_4 production induced by A23187 and AA in human neutrophils, in a concentration-dependent manner. The results point out to an inhibition in a range of 36 to 71%, at 20 μM, the highest tested concentration. The most active one was xanthone **1c** with a 2-catechol group presenting an IC_{50} value around 9 μM.

The 2,3-diarylxanthones were also tested for their ability to inhibit PGE_2 production stimulated by A23187 and LPS in human whole blood. At 10 μM concentration, xanthones **1a**, **1b**, **2a**, and **3a** (possessing at least one non-substituted aryl group) exhibited COX-1 inhibitory effects higher than 50% and with IC_{50} values from 1 to 7 μM. The COX-2 activity was unaffected by the 2,3-diarylxanthones, up to the highest tested concentration, 100 μM. In conclusion, the 2,3-diarylxanthones studied had a dual impact on the 5-LOX and COX-1 activities, an important contribution to reinforce the potential of this class of heterocyclic compounds in the modulation of the production of inflammatory mediators and it challenges to pursuit for novel structures with improved selectivity in the inflammatory cascade.

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